From the Children's Hospital, University of Basle
(Director: Prof. A. Hottinger)
Arbeit unter der Leitung von PD Dr. H. Berger

The Early Anemia of Prematurity

Therapeutic Trials with Cobalt

Inaugural-Dissertation
zur Erlangung der Doktorwürde der Medizinischen Fakultät
der Universität Basel

vorgelegt von
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von New York

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Introduction

The phenomenology of the early anemia of prematurity has been extensively discussed in several recent publications (*Betke*, *Gairdner* et al., and *Schulman*). The common etiological factors of low initial hemoglobin mass, i.e. low iron stores at birth (*Schulman*), and rapid rate of growth are common to both the early and the late anemias of prematurity. The hypothesis of a functional insufficiency of myeloid erythropoesis being the additional etiological factor of the early anemia of prematurity has lost some credibility since the publication of *Gairdner* et al., and *Seip* and *Halvorsen*'s extensive observations. A humoral regulatory mechanism may be suspected of being the primary factor. *Betke* assumes that this regulation is adequate to the needs of the organism for oxygen transportation. While probably true in the case of those hematological changes of the first three months of life in full-term infants which are analogous to the early anemia of prematurity, this assumption remains unsubstantiated in the case of the early anemia of prematurity.

The reduction of circulating hemoglobin and erythrocyte count comes to an end in normal full-term infants at the age of 8 to 10 weeks, having reached a minimum of 11.5 g% hemoglobin and 3.8 million erythrocytes, as an average. These values then begin slowly to rise. In premature infants, the reduction is more rapid, much lower minimal values being reached sooner (the early anemia of prematurity), and these values remain depressed longer (the late anemia of prematurity). *Gairdner* et al. have shown that there is a lag of about 2 weeks between the stimulation of the erythropoetic regulatory mechanism (by a fall in hemoglobin concentration to below 11 g%) and the erythropoetic response, as determined by the appearance of greater numbers of reticulocytes in peripheral blood, arrest of the decline in hemoglobin concentration and commencement of increase in hemoglobin mass. This is true for premature as well as full-term infants. The more rapid reduction of hemoglobin concentration, however, allows much lower levels to be reached by the premature infants during this lag period.

In premature infants, with the exception perhaps of the brain and skull, the growth of tissues remains permanently retarded in comparison with that of full-term infants *(Hallman)*. This leads to the suspicion that their energetic requirements for oxygen transportation are not met by the drastically low hemoglobin levels which they reach, and to the wish to treat or prevent their anemia.

Unlike the late, iron-deficiency, anemia of prematurity, the course of the early anemia remains unaffected by the prophylactic or therapeutic administration of iron *(Blackfan* and *Diamond, Gaisford* and *Jennison, Magnusson, Meritt* and *Davidson, Schulman, Wolff* and *Goodfellow)*. The usual treatment of severe cases is the transfusion of blood. *Rossier* and *Potiron* suggest beginning a course of transfusions early and repeating them often as a prophylaxis of the early anemia.

A number of recent reports have noted the successful use of cobalt *(Coles, Coles* and *James, Gairdner* et al., *Knütter, Kohler, Lindblad* and *Wegelius, Quilligan, Schmöger, Tevetoglu, Wegelius, Weippl,* and *Willi)*. Cobalt acts pharmacodynamically to produce a true and marked polycythemia, reticulocytosis, myeloid hyperplasia, and extramedullary erythropoetic activity. The hemoglobin formed under the influence of cobalt medication is not abnormal and has the normal capacity for oxygen transportation. These actions of cobalt occur, however, only in the presence of adequate amounts of copper and iron (*Rothlin* et al.).

Toxic effects of cobalt have led a number of authors to advise against its use except in rare circumstances and then only with extreme caution. In the cobalt therapy of infants the commonly reported toxic effect is the appearance of thyroid disorders. Because of toxicity *Dameshek* et al. concluded that "cobalt has little if any value in the clinical management of anemias".

Toxic effects have been most widely reported by those who have used cobaltous chloride, nitrate and sulfate. *Weissbecker, Goldeck,* and *Rothlin* et al. have shown that certain organic cobalt compounds are fully effective in antianemic therapy and are much less toxic.

In consideration of the promise which this controversial method of therapy offered to provide a prophylaxis of the early anemia of prematurity, we decided to gather some experience of our own in the use of cobalt in premature infants.

Method

The observations described in this present work concern a series of premature infants hospitalized at the University Children's Clinic, Basle, between the Fall of 1959 and the Spring of 1960. Their ages at admission varied between one hour and three days. Their weight at birth varied between 710 and 2450 grams, whereby most were between 1700 and 2400 grams. They were randomly divided into 3 groups, whereby average birth weight and length of all groups were approximately equal. The supportive therapy was identical for all groups. Until reaching about 2.5 kg they were given mother's milk to which ½% amino acid mixture *(Nesmida)* was added. After reaching this weight they received *Humana*, a cow-milk preparation which had been adjusted to resemble mother's milk by partial elimination of casein and calcium, and addition of lactose, dextrines and fats, as well as ascorbic acid (30 mg per liter), vitamin D_3

(560 I.U. per liter), and ferrous iron (0.83 mg per liter). Penicillin or achromycin plus B-vitamins were administered during the first few days according to the individual pneumonic findings. Oxygen, respiratory stimulants and circulatory stimulants were given as indicated in the individual case. All received vitamins K_1 and D_3 prophylactically. In addition, with the exception of one group, as will be discussed below, all received about 50 mg ascorbic acid daily. Hemoglobin, erythrocyte-count, and reticulocyte-count were determined weekly.

One group of 15 premature infants received *CCF*, a cobalt-copper-iron preparation from Sandoz containing, per dragee, 0.5 mg cobalt glutamate, 1.0 mg copper leucinate, and 22 mg ferrous gluconate. Beginning on the 15th day after birth, daily ¼ dragee was administered orally in small fractions distributed over the course of the day.

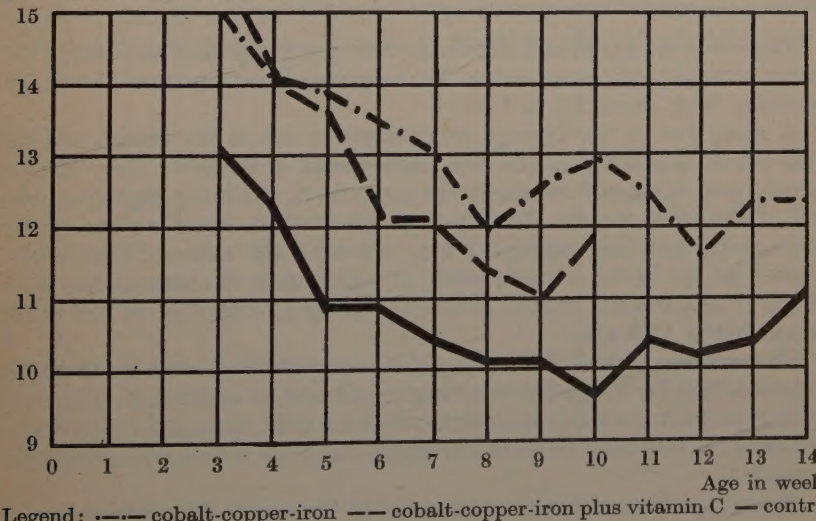
A second group of 15 premature infants received *no specific anti-anemic treatment.* Two years ago a similar study was carried out at this clinic by *J. Cohn*, who examined the effect of different milk-diets upon the anemia of prematurity. He found practically no difference in the average hemoglobin, erythrocyte count, color-index and reticulocyte count curves between a group of 24 infants fed exclusively with mother's milk, a group of 38 with Humana, and a group of 42 with 10% of a buttermilk preparation (Eledon) plus starch (Soldor) and dextrines and maltose (Nutromalt). We find no significant difference between the results with the present group without specific antianemic treatment and those of *Cohn*'s groups, and have averaged the results of these groups to obtain values representing a control-group of 119 infants.

In the course of our observations it became apparent that the results we obtained with the cobalt-copper-iron group, while somewhat better than those obtained with the other group, were rather less than those reported by *Weippl* who used slightly lower dosage of the same preparation. In consideration of the great variability of the results reported by various authors in the treatment of anemias with cobalt, this was not surprising. The question of the cause of this variability arose. Several observations led to the suspicion that ascorbic acid was the agent responsible. *In vitro*, cobalt forms a stable complex with carboxyl groups of ascorbic acid. *Barron* reported a lowering of the serum ascorbic acid level in cobalt polycythemias not occuring in other polycythemias. *Weissbecker* suggested that *Barron*'s determinations, obtained with dichlorphenol-indophenol reduction, show a lowering of the level of "active" ascorbic acid, that is ascorbic acid capable of hydrogen transfer, and do not say anything about the total ascorbic acid level. Does ascorbic acid in blood and tissues react with cobalt as it does *in vitro* forming an inactive complex? Or is the lowered ascorbic acid level a secondary effect of another action of cobalt? *Weissbecker* was able to temporarily hinder the cobalt polycythemia in experimental animals by administration of ascorbic acid. Atropine, however, prevents this action of ascorbic acid. Also, in spite of continued ascorbic acid administration, the hemoglobin, erythrocyte count, reticulocyte count, and the myeloid erythropoetic activity begin

slowly to increase after several days. *Weissbecker* explains this as an action of vitamin C preventing cobalt from causing a discharge of adrenalin which in turn would cause an emptying of depots of concentrated blood, producing an immediate polycythemia. Would the administration of greater (saturating) doses of ascorbic acid also prevent the erythropoetic action? This question prompted us to extend our observations. A third group of 35 premature infants were given the cobalt-copper-iron preparation (CCF) in the same dosage as the previous group, with the same supportive treatment except that they received no ascorbic acid apart from the small amounts contained in their milk-diet.

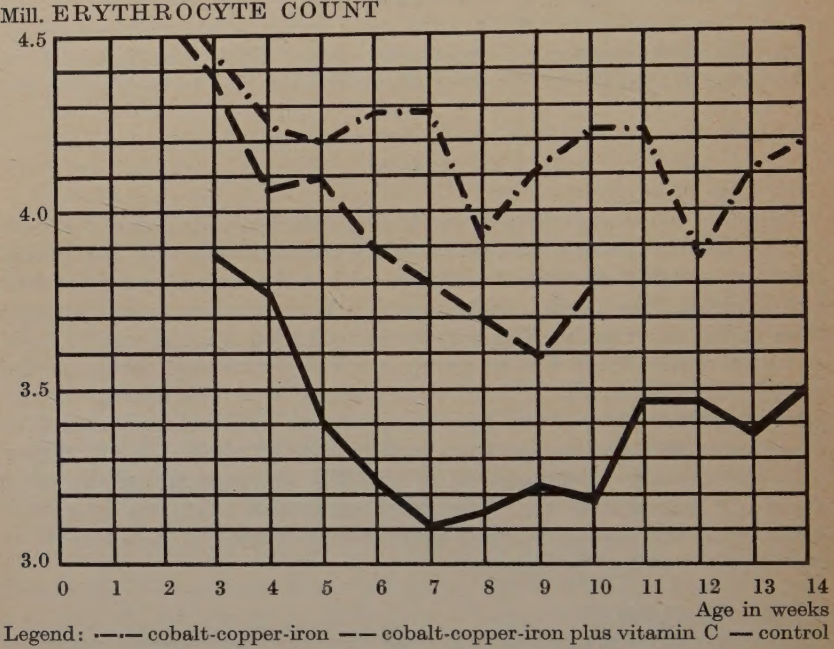
Results

Figure 1 is a comparison of the hemoglobin concentrations of groups with cobalt-copper-iron, and no specific antianemic therapy. The groups receiving cobalt remained at all times within the normal range for full-term infants. The decline in hemoglobin concentration was less rapid than in the other group. The lowest concentration reached was 11.5 g% at twelve weeks of age for one group, and 11.0 g% at eight weeks for the other group. From the fifth to the eleventh week the average hemoglobin concentration of the group receiving no additional vitamin C to that normally found in mother's milk, averaged about 2.2 g% higher than the average of the control group. The development of the early anemia of prematurity was prevented in almost all infants in the groups with cobalt medication. None in these groups showed the late anemia at the time of leaving the hospital.



Legend: ·—·— cobalt-copper-iron — — cobalt-copper-iron plus vitamin C — control

The course of the erythrocyte counts (figure 2) shows roughly the same relationships between the group average figures as in the hemoglobin curve. The average of the groups receiving cobalt remained consistently within the range of normal values for full-term infants. The average of the control group declined rapidly to reach a minimum of 3.12 million in the seventh week, and then began slowly to rise.



Legend: ·—·— cobalt-copper-iron —— cobalt-copper-iron plus vitamin C — control

There was no significant difference between the groups as regards the course of the average color-index. The average values remained above 1.0, declining from about 1.2 to 1.05.

A sharp rise in the average reticulocyte count of the groups treated with cobalt was noted in the first three weeks of therapy. The reticulocytosis then remained at about the same level, declining slightly at the end of the third month. The average reticulocyte count values of the other group rose less rapidly at first, reaching the value of the cobalt-treated groups in the seventh week, at which time the hemoglobin level of the cobalt-treated groups averaged 13.0 g%, and that of the other group, below 11.0 g%.

The average values for hemoglobin concentration and erythrocyte concentration for the group receiving cobalt and no additional vitamin C to that amount normally present in mother's milk remained consistently somewhat better than those of the group receiving cobalt and additional vitamin C.

No toxic effects of cobalt were noted in the course of treatment.

Conclusions and Summary

Low initial hemoglobin mass and rapid rate of growth are the common etiological factors to the early and the late anemias of prematurity. Whereas the late anemia is an iron-deficiency anemia, effectively treated or prevented by iron-administration; the early anemia is caused by an insufficiency of erythropoetic response to the stimulus of low hemoglobin level. Because the bone marrow itself is capable of greater activity, the insufficiency must be of the humoral erythropoetic regulatory mechanism. The opinion that the early anemia of prematurity is physiologic and the result of an active regulation adequate to the requirements of the organism, as the analogous condition in full-term infants quite probably is, was found to be without proof, and contrary to some of the available evidence.

We have been able to corroborate the positive results published in several recent reports on the prophylaxis of the early anemia of prematurity with cobalt. In view of the lower toxicity of the newer "organic" cobalt preparations, we feel that this method of treatment deserves greater attention.

Furthermore, we have found the antianemic effectiveness of cobalt-therapy to be diminished by the administration of ascorbic acid.

Zusammenfassung

Als Prophylaxe der Frühgeborenenanämie wurde in der vorliegenden Arbeit ein Kobalt-Kupfer-Eisen-Präparat verwendet. Es wurde nicht nur die zweite Phase der Frühgeborenenanämie, sondern auch die sonst refraktäre erste Phase erfolgreich verhütet. Keines der Kinder hat toxische Zeichen gezeigt. Es wurde bemerkt, daß die Wirksamkeit dieses Mittels durch Ascorbinsäurezugabe in negativem Sinne beeinflußt wurde.

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